ABSTRACT OF THE INVENTION

METHODS FOR IDENTIFYING AN ESSENTIAL GENE IN A PROKARYOTIC MICROORGANISM

Methods are provided for the rapid identification of essential or conditionally essential DNA segments in any species of haploid cell (one copy chromosome per cell) that is capable of being transformed by artificial means and is capable of undergoing DNA recombination. The BAC system is used to provide to the prokaryotic host cell an additional copy of a known segment of DNA of the host cell (or of another prokaryotic cell whose genome is known) to construct merodiploid test cells wherein the chromosomal region of the host cell that is homologous to the DNA contained in the BAC becomes diploid. Alternatively, due to the high homology in essential genes of prokaryotes, the DNA contained in the BAC can be derived from a prokaryote other than the host cell. A transposon is then delivered randomly to the merodiploid cell. Due to presence of the BAC carrying a segment of homologous DNA, the merodiploid cell will survive replication if the transposon disrupted gene on the host chromosome is replaced by a second normal gene existing on the particular BAC contained within the particular host cell. However, in the event the BAC carrying the second normal gene copy is lost during replication or the BAC replaces a normal gene in the host cell with a defective copy due to recombination, inhibition of growth or lethality of the test cell will result. This system offers an enhanced means of identifying essential function genes in diploid pathogens, such as gram-negative and gram-positive bacteria.